Table 1

Item	USP 4,897,173	The present invention
1. General feature constitution	Base plate, reaction layer and carbon electrode system.	Base plate, reaction layer, carbon electrode system, cover, and space surrounded by these members.
Reaction	Reaction of enzyme, electron acceptor and sample solution is effected in open space.	Reaction is effected in the surrounded space.
2. Material on electrode	Protein/electrode	Hydrophilic polymer/electrode
3. Method for preparing electrode	Carbon electrode is prepared by a method comprising a printing step and a drying step under heating.	Carbon electrode is prepared by a method comprising a printing step, a drying step under heating, and a step of coating the electrode thus formed with a hydrophilic polymeric layer tightly adhered to the electrode.
4. Reagent	Enzyme and electron acceptor are supported on porous material disposed in cavity in frame. Porous material is present on electrode	Reaction layer consisting of enzyme, electron acceptor and hydrophilic polymeric material are formed so as to tightly adhere onto carbon electrode surface. Porous material is not used.
5. Cover	Resin cover is disposed on upper portion of porous material. Cover has hole at a position above upper portion of electrode.	Cover is disposed over reaction/electrode so as to hold the space. Cover has no opening above upper portion of electrode. Porous material is not used.
6. Enzyme	GOD is supported on porous material.	GOD is tightly adhered onto carbon electrode surface.
7. Electron acceptor	Potassium ferricyanide is supported.	Potassium ferricyanide is tightly adhered onto carbon electrode surface
8. Filtration membrane	Polycarbonate membrane is disposed on upper portion of reaction layer.	No filtration membrane is used.

From the table given above and also from Figs. 3, 4, 8 and 9 of this reference, it is observed that the space, which is present in the biosensor according to the invention, is not disclosed in Nankai et al. According to the teaching of Nankai et al., the porous material, which supports GOD and an electron acceptor, is disposed in a space on the electrode. A resin cover is disposed on the space so as to coincide the position of the hole in the cover with the position of the porous material. Thus, the space is upwardly open in view of the positional relationship between the cover and the electrode. In Fig. 8 of Nankai et al., there can be observed a space formed by frame 17 and plate 22, but this space is present on the electrode. The upper portion of the electrode is substantially covered with the liquid-retaining-layer. According to the teaching of Nankai et al., a sample solution is dropwise supplied from the upper portion above the electrode. So, if there is a gap on the electrode and if the amount of the sample solution is small, then it will be difficult for the sample solution to arrive at the electrode. It is therefore necessary to dispose members, such as liquid-retaining-layer, above the electrode to adequately guide the sample solution. If a porous member is disposed above the electrode, a replacement of air with liquid will not be satisfactorily effected, so that it is probable that undesired air bubbles are generated. Such air bubbles may cause a disturbance of the response current.

Contrary to the constitution disclosed in Nankai et al. as explained above, the biosensor according to the invention has the following characteristic features:

(1) A space is present above the electrode with the proviso that the space is surrounded by the base plate of the electrode and by the cover which is situated opposite to the base plate.

(2) The reaction layer is formed in such a manner that the reaction layer is tightly adhered to the electrode surface.

(3) The members, such as the liquid-retaining-layer and the porous material disclosed in the reference, which fill the space, are not employed in the biosensor according to the invention.

(4) The present biosensor has an inlet for the sample solution, and an outlet for air.

Owing to the constitution mentioned above, the biosensor according to the invention has the following remarkable effects:

(1) Only by contacting a sample solution with the inlet for said solution, is it possible to pass the sample solution to the space by means of capillary action.

(2) The space is not filled with a liquid-retaining-layer and other members, and the reaction layer is tightly adhered to the electrode surface, so that a sample solution is smoothly introduced so as to fill the space above the electrode without generation of air bubbles.

(3) The members, which are open to the external space, are only the outlet and the inlet. The space, which is present above the electrode, is surrounded by the spacer and the cover. Therefore, in the course of a measurement operation, a

concentration change of the sample solution due to water evaporation is negligibly small.

(4) For the measurement, it is required to use a sample solution only in a small volume nearly equal to that of the space. Thus, the volume of the sample solution required is very small.

Furthermore, the Examiner has not compared the claimed invention with the teachings of Nankai et al. The Examiner only sets forth the teachings of Nankai et al. but does not discuss the differences between Nankai et al. and the claimed invention nor provides any reason why one having ordinary skill in the art would find it "obvious" in the sense of 35 USC §103 to modify Nankai et al. to arrive at the claimed invention. In view of the fact that the biosensor, according to the invention, has very good properties and that the construction and effect of the present biosensor is neither taught nor suggested in Nankai et al., Applicants respectfully submit that Nankai et al. fail to establish a *prima facie* case of obviousness for the claimed invention. Accordingly, withdrawal of the rejection is respectfully requested.

It is noted that Nankai et al. is used as a primary reference in combination with one or more secondary references in further rejections of the claims.

For example, claims 1-5, 7-10 and 14 stand rejected under 35 USC §103 as being unpatentable over Nankai et al. in view of Otagawa et al. Nankai et al. has been discussed hereinabove. The differences between the present invention and Otagawa et al. are discussed below.

(1) Re: the term "gap"

The term "gap", as used in Otagawa et al., means the gap between the electrodes formed on the base plate. In the gap between the electrodes, the electrolyte bridges over the electrolytes. In Fig. 2 of Otagawa et al., it is observed that the barrier 42 is tightly adhered to the outfacing surface 24. Thus, Otagawa et al. does not disclose nor suggest any space corresponding to the space present in the biosensor according to the invention.

In Fig. 1 of Otagawa et al., there is disclosed the counter-electrode 30 which seems to cover the upper portion of the other electrode. This feature is greatly different from the arrangement feature of electrodes disposed in the biosensor according to the invention. In column 6, line 23ff of the reference, it is stated that the gap 34 is between the electrode and the counter-electrode. From this statement and Fig. 5 of the reference, it is observed that the three electrodes are disposed on one and the same plane, and that the electrolyte layer and the barrier layer are tightly adhered to these electrodes. The barrier layer is disposed in the outermost position. The reference does not disclose a space corresponding to the space present in the biosensor according to the invention.

Furthermore, in column 6, line 45 of Otagawa et al., it is stated that the barrier 42 is gas permeable but aqueous-solution impermeable. When soaked in a liquid, the barrier inhibits any passage of the sample liquid into the internal portion of the sensor, while only the aimed gas is allowed to pass. Referring to this point, it is stated in column 11, line 65ff of Otagawa et al. that the barrier 42 is impermeable to the electrolytic medium

28 to prevent escape and/or mixing with any analyte solution exterior of the barrier 42.

On the other hand, the biosensor according to the invention is so designed as to smoothly introduce a sample solution into the space above the electrode. Thus, the invention is quite different from Otagawa et al. which disclose a structure for the prevention of liquid permeation.

As explained above, the "gaps", disclosed in Otagawa et al., are greatly different from the "space" present in the biosensor according to the invention. The "space" is neither disclosed nor suggested by Otagawa et al.

(2) Re: the term "hydrophilic polymer"

Otagawa et al. discloses the use of a hydrophilic polymer. However, it should be noted that, according to the teaching of this reference, hydrophilic polymers are used as gel for the electrolytic medium, and the nature of the hydrophilic polymers are maintained by supplying water thereto from the aqueous reservoir, so that humidity-independent sensor responses are obtained.

In column 7, lines 12-19 of Otagawa et al, there are disclosed the passages "The aqueous reservoir can be included ..... to keep the electrolytic medium from drying out" and "Such aqueous reservoirs can be used in conjunction with all embodiments of the invention." Thus, the reservoirs are indispensable for the sensor disclosed in Otagawa et al.

In the case of the biosensor according to the invention, hydrophilic polymers are used, for instance, for the purpose of avoiding any influence of solid components on the carbon electrode. Furthermore, the reaction layer, which contains the hydrophilic polymers, is present in a dried state. "Aqueous

reservoirs" are quite unnecessary in the biosensor according to the invention.

(3) Re: the term "membrane"

The Examiner mentions that membranes, made of carboxy-cellulose, gelatin, methylcellulose, vinyl alcohol and polyvinyl pyrrolidone, are disclosed in Otagawa et al. However, in column 11, line 36ff of Otagawa et al., it is stated that the polymers are gels contained in the electrolytic medium. The electrolytic medium is contacted with the aqueous reservoir in order to avoid any drying out thereof (Figs. 2 and 10 of Otagawa et al.). The reason why the aqueous reservoir is necessary in the sensor disclosed in the reference, is that the humidity causes a change of the characteristic properties of the electrolyte, although it is immaterial as to whether the humidity causes a change of the concentration of the gaseous components *per se*.

In the case of the biosensor according to the invention, hydrophilic polymers are supported together with the enzyme and the electron acceptor in the dry state. The evaporation of water is suppressed by introducing a sample solution onto the electrode disposed in the space surrounded by the cover. In this manner, any concentration change of the components contained in the sample solution may effectively be suppressed.

Thus, it is clear that Otagawa et al. do not correct the deficiencies of Nankai et al. nor has the Examiner established any reason to motivate the ordinary worker skilled in the art to modify Nankai et al. in view of Otagawa et al. Applicants respectfully submit that the rejection fails to establish a *prima facie* case of obviousness and withdrawal thereof is respectfully requested.

Claims 13 and 15-17 also stand rejected under 35 USC §103 as being unpatentable over Nankai et al. and Otagawa et al. (presumably for the reasons as applied to claims 1-5, 7-10 and 14) and further in view of Higgins et al. This rejection is respectfully traversed.

The deficiencies of Nankai et al. alone or in view of Otagawa et al. have been discussed above which comments are herein incorporated by reference.

Higgins et al. disclose an enzyme electrode, wherein use is made of carbon and metals. The carbon is "solid carbon" or "carbon rod". The mediators, described in the reference, are water-insoluble compounds such as ferrocene and derivatives thereof and chloranil, etc. Furthermore, this reference mentions that these compounds are used in the form of their polymers which are difficultly soluble. The enzymes are immobilized or are made insoluble. This is described in column 4, line 13ff of Higgins et al.

Furthermore, in the sensor disclosed in Higgins et al., use is made of a dialysis membrane in order to avoid any loss caused by the leaching of enzymes and mediators. These features serve the main purpose of the sensor disclosed in this reference, because the sensor is aimed at the use as "an *in vivo* sensor".

On the other hand, the biosensor according to the invention is so designed that the hydrophilic polymers, enzymes and electron acceptors, supported on the electrode, are swiftly dissolved in a sample solution, and that the enzyme reactions proceed quickly. So, it is quite unnecessary for the present biosensor to employ any means for suppressing a leaching as disclosed in Higgins et al.

Furthermore, by the use of the biosensor according to the invention, it is possible to determine a substrate concentration in a simple manner including only the dropwise supply of a sample solution. The biosensor is a sensor of "single use", so that it is impossible to previously effect a calibration of individual sensors with the aid of a glucose standard solution. So, the individual sensors should have uniform and stable response performances. If enzymes are immobilized on the electrode or if mediators are coated so as to form an insoluble or difficultly soluble membrane according to the teaching of Higgins et al., then it is necessary to adjust the thickness of the membrane and also to adjust the diffusion properties of the substrate contained in the membrane in order to adjust the response performances. This is very difficult work, and provides a disadvantage in the production of the sensors on a large scale. On the other hand, in the case of the biosensor according to the invention, the enzymes and the electron acceptors are supported on the porous materials, and these substances are dissolved in a sample solution, so that it is possible to produce the biosensors having uniform characteristic performances. This is an unexpected benefit of the invention. In column 30ff of Higgins et al., a description is given for disposable sensors, but the reference lacks any concrete explanations of the structure of such disposable sensors.

As stated above, the reference is greatly different from the invention. The reference does not contain any descriptions which suggest the biosensor according to the invention nor a method for producing the same.

For the foregoing reasons, withdrawal of the rejection is respectfully requested.

Claims 13 and 18 also stand further rejected under 35 USC §103 as being unpatentable over Nankai et al. and Otagawa et al. further in view of Newman et al. This rejection is also respectfully traversed.

Newman et al. disclose a membrane comprising three layers, namely a first layer, such as a silicone layer, a second layer, such as a porous layer, and an enzyme layer positioned between the first and second layers. This membrane is water-insoluble. Therefore, it is impossible to use the membrane for the preparation of a disposal sensor as in the case of the present invention.

In the case of the biosensor according to the invention, the enzymes and the electron acceptors are supported on the porous materials in the dry state. These substances are dissolved in a sample solution, so that the biosensor have uniform characteristic performances. This is a characteristic feature of the invention.

If use is made of an insoluble membrane as disclosed in Newman et al., then it would be necessary to adjust the membrane thickness and the diffusion properties of the substrate contained in the membrane in order to adjust the response performances. This is very difficult work, and provides a disadvantage in the production of the sensors, having the same characteristic performances, on a large scale.

The cell disclosed the reference comprises:

a chamber,

an electrolyte (liquid solution),

an electrode, and

a membrane.

The electrolyte, in the form of "a liquid mixture of electrolyte", is an indispensable component of the known cell, as mentioned in the description given in column 4, line 51ff of Newman et al.

On the other hand, the biosensor according to the invention has a reaction layer, which is in the dry state, adhered to the electrode surface. In this point, the invention greatly differs from Newman, et al. Namely, this feature is not suggested in the reference.

Thus, Newman et al. neither teaches the limitations of claims 13 or 18 nor does it cure the deficiencies previously discussed in the combination of Nankai et al. and Otagawa et al. In view of the foregoing comments, withdrawal of the rejection is respectfully requested.

Applicants also note the citation of Oberhardt as being "pertinent to Applicant's disclosure" although not applied in a rejection of the claims.

Oberhardt discloses a multilayer membrane and a method for producing the same. This membrane comprises:

> dense layer/less dense layer/enzyme layer/less dense layer. The membrane is water-insoluble as in the case of USP 3,979,274 by Newman et al. The cell, disclosed in the reference (USP 4,418,148 by Oberhardt), is composed of the water-insoluble membrane, an electrolyte, a chamber and electrode means as in the case of USP 3,979,274 by Newman et al. Furthermore, in column 9, line 11ff of the reference (USP 4,418,148 by Oberhardt), it is stated that the electrolyte is "a liquid mixture of electrolyte".

Thus, Oberhardt is greatly different from the biosensor according to the invention with respect to the constitution of the membrane and cell.

In order to summarize the deficiencies of the prior art in their teachings as compared to the present invention, Applicants present the following Table 2 for the Examiner's consideration.

Table 2

INVENTION, PRIOR ART	Main features		
	cell component		reagent
The present invention	•anode •cathode	•space •(cover, introducing port, discharge port)	•enzyme (dried, soluble) •electron acceptor (dried, soluble) •(formed on the electrodes)
USP 4897173 Nankai et al.	•anode •cathode	-------	•enzyme (dried, soluble) •electron acceptor (dried, soluble) (carried on porous material)
USP 4900405 Otagawa et al.	•anode •cathode	•aqueous reservoir •electrolyte •barrier	----------
USP 4545382 Higgins et al.	•anode •cathode	•membrane (insoluble)	•immobilized enzyme •mediator (insoluble)
USP 3979274 Newman et al.	•anode •cathode	•chamber •electrolytic solution •membrane (insoluble)	•immobilized enzyme
USP 4418148 Oberhardt	•anode •cathode	•chamber •electrolytic solution •membrane (insoluble)	•immobilized enzyme

As to the above table relating to the comparisons, the following comments are added:

(1) The "chamber", shown in the references by Newman et al and by Oberhardt, is not a sample chamber, but a chamber used for supplying electrolytes (liquid solutions) to the internal portion of the electrode. This is different from the "space" present in the biosensor according to the invention. If use is made of the electrolytes in the dry state in the apparatus disclosed in the two references, then it is not probable that the enzyme reactions will proceed immediately after a sample solution has been introduced into the apparatus.

(2) The "space" is present only in the biosensor according to the invention. Due to this feature, a sample solution can be smoothly introduced into the space by a simple operation which only comprises contacting the sample solution with the inlet port for the introduction. In addition, the evaporation of water during the measurement operation can effectively be suppressed.

In view of the foregoing comments, it is clear that the constitution and operation of the biosensor according to the present invention would not have been obvious to the ordinary worker skilled in the art at the time the invention was made. It is only through the benefit of Applicants' disclosure, which the Examiner utilizes as a guide in selecting bits and pieces of the prior art, which provides any suggestion to modify the teachings of the prior art in an attempt to teach the claimed invention. In view of the great differences of the present invention from the teachings of the references, not only with respect to its constitution, but also the operational mode of the sensor, Applicants respectfully submit that the claimed invention would